Cocaine-induced inheritable epigenetic marks may be altered by changing early postnatal fostering

Ajonijebu, Duyilemi C.; Abboussi, Oualid; Mabandla, Musa V.; Daniels, William M. U.

Published in:
NeuroReport

DOI:
10.1097/WNR.0000000000001332

Publication date:
2019

Document Version
Peer reviewed version

Cocaine-induced inheritable epigenetic marks may be altered by changing early postnatal fostering

Duyilemi C. Ajonijebu[a,b], Oualid Abboussi[c], Musa V. Mabandlaa and William M.U. Daniels[d]

[a]Discipline of Human Physiology, School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban,

[b]Department of Physiology, School of Biomolecular and Chemical Sciences, Faculty of Science, Nelson Mandela University, Port Elizabeth, South Africa,

[c]Institute of Academic Anaesthesia, Division of Neuroscience, Ninewells Hospital and Medical School, University of Dundee, Dundee, UK

[d]School of Physiology, University of the Witwatersrand, Johannesburg, South Africa.

Correspondence to Duyilemi C. Ajonijebu, PhD,
Nelson Mandela University, Port Elizabeth 77000, Eastern Cape, South Africa
Tel: +27 (0) 61 8723924; fax: +27 (0) 41 504 2814;

Abstract

Here, we explored the hypothesis that parental cocaine exposure could alter epigenetic machinery in their drug- naive offspring while early postnatal fostering may further modify the accompanied neurochemical and functional components. Variant drug-naive pups were produced from cocaine-exposed or unexposed C57BL/6 female mice that were matched with their male counterparts for mating. Within 3 days of birth, half of the pups were cross-fostered and nurtured by non-biological lactating dams. The pups were initially examined for locomotor activity and memory performance and subsequently for changes in DNA methylation in promoter regions of cAMP response element modulator (Crem) and Fosb in the prefrontal cortex at 48 days postnatum. The impact of postnatal fostering on these parameters was also investigated. Our results showed that cocaine exposure significantly decreased both Crem and Fosb methylation in the prefrontal cortex of progenitor mice, while similar patterns of methylation were replicated in the brains of drug-naive non-fostered offspring mice but reversed by postnatal fostering. Furthermore, offspring raised by cocaine-exposed dams were impaired in discriminative learning and exhibited memory decline, whereas locomotor activity remains unaltered in all groups of mice. Our data provide some evidence that indirect exposure to cocaine may cause marked epigenetic changes within the cortical networks of drug-naive descendants and that mediation by Crem/Fosb signalling in this brain region may be beneficial, while early postnatal fostering may further engineer molecular switching that may predispose the individual to future risky behaviours as well as accumulative potential to developing cognitive impairment later in life.

Keywords: cocaine, DNA methylation, early postnatal fostering, epigenetic inheritance, sex differences.

Introduction

Despite evidence for epigenetic inheritance of gene profiles, the exact molecular and circuit-linked regula- tory mechanisms that underlie behavioural responses to drug-taking, the transfer of drug-induced epigenetic marks to succeeding generations and how offspring of addicted and non-addicted drug-taking parents are pro- grammed to exhibit some of these transferred traits, are not fully understood. Information about these phenom- ena may contribute significantly to expanding our under- standing of the molecular underpinnings of drug abuse and its accompanied heritable impact.

DNA methylation represents an important regulatory mechanism that induces long-term stable changes in gene expression patterns. These patterns may be deter- mined by factors external to the body, for example, envi- ronmental factors [1] and may subsequently influence the behaviour of the individual [2]. Studies have demon- strated that DNA methylation patterns may also be transgenerational under certain circumstances including substance abuse [3]. Interestingly, it has been postu- lated that maternal DNA methylation status is the main determinant of inheritance of parental phenotypes by the offspring, possible due to escape of epigenomic repro- gramming at gametogenesis [4]. In support of this view, it has been shown that maternal exposure to high doses of cocaine in the second and third trimester of gestation caused profound alterations in global DNA methylation in offspring mice with evidence of structural and func- tional modifications in the postnatal brain [5]. In contrast, there is also evidence that indicates a contribution of the male epigenome in transgenerational inheritance of epi- genetic traits [6]; however, the impact of paternal effects on the phenotype of the offspring may be modulated by maternal behaviours [7]. However, such modulation may not always be beneficial to the offspring. For instance, the interaction between adverse early life experiences with the epigenome, and its potential to drive vulnera- bility towards the development of mental illness, has recently been documented by Lewis and Olive [8]. A study showing children that were repetitively maltreated to have significantly greater methylation in the promoter region of the glucocorticoid gene provided further evi- dence for the link between childhood stress and epi- genetic changes [9]. Social changes in the environment especially during early life may therefore have a massive influence on the epigenetic make-up of an individual [10], and these changes in DNA methylation status with consequent effects on gene expression may alter behav- iour significantly [11].

In a previous study, we demonstrated that voluntary oral consumption of cocaine was associated with charac- teristic patterns of methylation in the promoter regions of Crem (cAMP response element modulator) and Fosb genes within the prefrontal cortex and hippocampus of C57BL/6 mice [12]. Additional to the established roles of Crem and Fosb in drug reward and vulnerability [13–15] is that they also mediate gamete functions [16] and nurtur- ing effects [17] in mice. These data led us to investigate first whether parental cocaine exposure could affect DNA methylation in specific promoters of Crem and Fosb genes in the prefrontal cortex of drug-naive offspring mice; we chose this brain region because it has been critically implicated in cocaine abuse as well as memory function [18,19]. Second, we assessed whether the expected epi- genetic changes are maternally or paternally inherited. Third, we examined whether the transmitted epigenetic

changes can be altered by early postnatal fostering and fourth, we determined whether these changes paralleled with memory performance and locomotor behaviour of drug-naive offspring mice.

Experimental procedures

Animal handling and treatment

This study was performed using C57BL/6 mice obtained from the Biomedical Resource Unit, University of KwaZulu-Natal, Durban, South Africa and housed in cli- mate-controlled rooms (21–23°C, 60% humidity) where they had free access to food and water. Female mice (dams, n = 16) were placed in free-living conditions in the IntelliCage (TSE Systems GmbH, Bad Homburg, Germany) that allows automated long-term tracking and behavioural phenotyping [20]. The mice were housed under these conditions for 58 days, where they had access to drinking bottles containing either 300 mg/L cocaine or water. Female mice (n = 8) in an Intellicage where all drinking bottles contained water, served as controls. The brains of four animals from each group (cocaine exposed and control) were collected for subsequent assessment of DNA methylation status. A separate four animals per group served as maternal progenitors during mating.

Male mice were housed and treated differently to females and were not placed in an Intellicage system. Male counterparts (sires) either received alternate intra- peritoneal injections of 10 mg/kg cocaine (n = 8) or sterile 0.9% saline (n = 8) for 11 days. Two males from each group served as paternal progenitors and were sub- sequently used for mating to produce the F1 generation of drug-naive offspring mice (Fig. 1a). The reason for not placing male mice in the Intellicage was that male mice with their innate social hierarchy nature have the pro- pensity to develop excessive aggressive behaviour under such conditions [21]. Also, our chosen treatment regimen for the F0 sires was supported by recent evidence that acute intraperitoneal injection of moderate dose of cocaine was able to elicit changes in F0 and F1 sperm methylomes [22].

Mating protocol

The mating groups were as follows: two control female mice and a control male mouse formed the non-drug exposed parent mice group (NEP group), two females exposed to drug paired with a control male to form the FE group, a drug-treated male paired with two control females became the ME group, a drug-treated male and two drug-exposed females paired to form the MFE group. Therefore, in all mating groups, two nulligravid dams were paired with one male in the same cage (Fig. 1b). Following successful mating the pregnant dams were separated and housed in different cages until delivery. The male mice were never in contact again with both the pregnant dams and their pups after birth.

Cross-fostering protocol

Within 3 days of birth, the entire litter of one of the treated dams in FE and MFE groups were cross-fostered in exchange for pups from the inception-matched dams in NEP and ME groups, respectively, whereas other primiparous dams retained their pups and reared them until weaned

at postnatal day (PND) 21. The weaned pups were reared in groups of four to six animals (same sex) per cage until young adulthood. Both non-fostered and fostered pups were subjected to subsequent behav- ioural testing.

All animal procedures complied with ARRIVE guide- lines and were approved by the University of KwaZulu- Natal's Animal Ethics Committee (approval number AREC/071/015) in accordance with National Institute of Health guidelines for the care and use of laboratory animals.

Memory function and locomotor tests

At PND 44–47, memory functioning and locomotor activity of the offspring were assessed using novel object recognition (NOR) and open field (OF) tests, respec- tively. NOR test is based on the premise that novelty elicits approach behaviour in rodents [23]. Before test- ing, each mouse was first habituated to the testing arena by being placed in a transparent box for 1 minute and later returned to its home cage. NOR tasks included two sessions (familiarization and test) which were performed as previously described [24]. In our protocol, two iden- tical objects (A + A) were first presented to the animals to explore in 5 minutes. Twenty-four hours later, one of the objects A (familiar) was replaced with object B (novel). Object exploration was later scored from the video recordings by a blind experimenter and expressed as the average time (in seconds) the mice spent exploring either the familiar (Tf) or the novel (Tn) object. The percentage time taken to discriminate the novel object in the 5-minute test session was calculated as a measure of recognition memory in the mice, using this mathematical expression: $[(T_n/T_n + T_f) \times 100]$ [25].

Because the NOR test required some form of physical activity, we needed to assess the locomotor status of each animal. For the OF test, we used a locally made black plexiglass (50 × 50 × 25 cm) apparatus. The floor was divided into 16 equal sized squares. Locomotor activity was assessed by recording the total number of squares crossed using a video camera affixed to a wooden holder positioned just above the apparatus.

Brain sample collection, DNA methylation and quantitative PCR

Both the parent mice and their variant offspring (at PND 48) were sacrificed. After removing the brains, prefrontal cortical tissues were dissected out on ice followed by DNA extraction (ZR Genomic DNA-Tissue MiniPrep; Inqaba biotec, South Africa) and bisulfite conversion (EZ DNA methylation kit; Zymo Research , USA), performed according to the manufacturer's instructions. Methylated DNA was then subjected to quantitative real-time PCR (qPCR) on LightCycler 2.0 (Roche Diagnostics (Pty) Ltd., South Africa) using the following primers: Fosb: TATAGAAGCGCTGTGAATGGA (forward), GACCATCTCCGAAATCCTACA (reverse), Crem: CAGAGGAAGAAGGGACACCA (forward), TTGTAT TGCCCCGTGCTAGT (reverse) and GAPDH: GCCAA AAGGGTCATCATCTCCGC (forward), GGATGACC TGCCCACAGCCTTG (reverse). The PCR reaction mixture consisted of 5 µL DNA template, 10 µL SYBR Green I (Roche Diagnostics , South Africa), 2 µL each of 15 µM primer pair stock and 1 µL DNA free water. Cq values were chosen within linear range while differences in methylation between

samples were determined using comparative Cq method as previously described [15,26].

Statistical analysis

GraphPad Prism software (version 5) was used for the statistical analysis. At first, we tested all data for Gaussian distribution using Shapiro-Wilk normality test. Where distribution was normal, data were analysed using Student's t-test or one-way analysis of variance followed by Bonferroni's comparison post-hoc test. If not, non-par- ametric analysis using Kruskal–Wallis test (comparison by Dunn's Multiple post-test) was used. All data are expressed as the mean ± SD with level of significance set at $P < 0.05$.

Results

DNA methylation changes in the prefrontal cortex of parent mice post-cocaine exposure

At first, we compared the effects of cocaine administered through oral (females) and intraperitoneal (males) routes on DNA epigenetic modifications in the prefrontal cortex of the parent mice. Our data showed that cocaine expo- sure, either by free choice access in dams or by repeated alternate intraperitoneal injections in the males, both resulted in significant DNA hypomethylation in the pro- moter regions of Crem (Dams: $t_5 = 29.19$, $P < 0.0001$; Sires: $t_6 = 30.22$, $P < 0.0001$, Fig. 2a) and Fosb (Dams: $t_6 = 34.59$, $P < 0.0001$; Sires: $t_6 = 32.20$, $P < 0.0001$, Fig. 2b) in the prefrontal cortex when compared to their respective controls. Sexual dimorphic effects on DNA modification was only significant for Crem methylation, indicating that the degree of hypomethylation in Crem promoter region was significantly more decreased by cocaine administration in female mice compared to males ($t_5 = 10.12$, $P = 0.0002$, Fig. 2c). No significant sexual difference was observed in methylation status in FosB promoters within the prefrontal cortex of the gender groups ($t_6 = 1.689$, $P = 0.1422$, Fig. 2d).

The patterns of DNA methylation expressed in the prefrontal cortices of drug-naive non-fostered offspring mice are similar to their progenitors

We next examined the prefrontal cortex of variant drug-naive offspring mice for methylation changes in the promoters of Crem and FosB that may be associated with paternal and maternal cocaine-imprints. We found that Crem methylation was significantly decreased in all the pup groups where at least one of the parents were exposed to cocaine (ME, FE, MFE), compared to the NEP group where none of the parents were exposed to cocaine ($F_{3,21} = 187.6$, $P < 0.0001$, Fig. 3a). qPCR assess- ment further showed that DNA methylation changes in FosB promoter region in the prefrontal cortex of the pups were similarly reduced ($F_{3,15} = 74.02$, $P < 0.0001$, Fig. 3b). These data indicate that cocaine-induced epigenetic imprints in naive offspring mice are similar to that of their cocaine-experienced progenitors.

Fostering alters expression of inherited cocaine imprints

To investigate the impact of early postnatal fostering and nurturing medium on epigenetic

signatures associated with cocaine exposure, we cross-fostered pups from NEP/FE groups and ME/MFE groups, respectively (Fig. 4a), at early life, so that all the offspring were raised by non-biological dams with or without cocaine experience. Epigenetic modifications of these animals were compared to those of their non-fostered littermates. The data indicate that cocaine-induced epigenetic imprints that were previously expressed within the brains of non-fostered offspring were different in the fostered off- spring (Fig. 4b and c).

We observed that the epigenetic changes in both Crem ($F_{3,20}$ = 3.304, P = 0.0455, Fig. 4b) and FosB (K statistics: K_4 = 6.283, P = 0.0986, Fig. 4c) promoters in the prefrontal cortex of fostered offspring tended towards hypermethylation, although these epi- genetic changes did not differ statistically from their controls (P > 0.05).

Drug-naive offspring mice raised by either biological or non-biological mothers exposed to cocaine are memory-challenged

We examined memory competence using the NOR testing paradigm in drug-naive offspring mice that were either reared by their biological (non-fostered) or non-biological (fostered) dams. Our findings show that both NEP and FE non-fostered offspring mice and their fostered counterparts attained above 50% discrimination index (Fig. 5a and b). This implies that non-fostered mice (NEP and FE) explored novel objects more than the familiar ones, and this exploratory behaviour was not altered by fostering the mice (t_{10} = 1.286, P = 0.0653, Fig. 5a; t_{10} = 1.219, P = 0.2509, Fig. 5b). Our data further indicated that ME non-fostered mice (nurtured by their biological drug-un-exposed dams) obtained an average discrimination score of 70%, but those ME offspring raised by cocaine-ex- posed fostered dams became memory-challenged such that they explored novel objects significantly less than the familiar objects when compared with their non-fostered counterparts (t_8 = 2.352, P = 0.0465, Fig. 5c). Surprisingly, MFE offspring in both non-fostered and fostered categories attained less than 50% memory score (t_{10} = 0.0983, P = 0.9236, Fig. 5d), indicating severe impairments in recognition memory of these offspring type irrespective of being fostered (raised by non-biological, drug-unexposed dams) or not (raised by biological cocaine-exposed dams).

Locomotor behaviour of drug-naive offspring mice

It is well known that direct exposure to cocaine alters locomotor activities in animals [27,28], but the impact on succeeding generations naive to cocaine remains unknown. We next investigated locomotor behaviour of the variant offspring mice of both cocaine-exposed and non-exposed parents. These offspring mice were either non-fostered or fostered before introducing them to the OF arena for locomotor activity scoring. We found that the total number of squares crossed were similar across all groups investigated. This observation indicated that neither parental cocaine exposure nor fostering after birth affected locomotor behaviour of the offspring mice ($F_{3,21}$ = 0.2457, P = 0.8633, Fig. 6a; $F_{3,23}$ = 0.5355, P = 0.6633, Fig. 6b).

Discussion

In this study, we compared the effects of voluntary cocaine consumption in free-living conditions (females) and intra- peritoneal cocaine administration (males) on epigenetic changes in certain brain areas with reference to sexually dimorphic effects. We observed that cocaine acquisition by the progenitor mice was associated with hypomethylation or transcriptional activation of Crem/Fosb in the prefrontal cortex, irrespective of the routes of administration. Interestingly, the difference in the extent of hypomethylation between the two sexs suggests that female mice may be more sensitive to the effects of cocaine than their male counterparts. Our observation is in line with the findings of a previous study by Zakharova et al. who showed that female rats are more sensitive to cocaine rewards than males. However since our experiments were performed at a stage when the animals were still in their reproduction age, the observed sex differences in cortical Crem profiles may also be due to influ- ences by gonadal hormones as previously posited [30]. The different methods and duration of cocaine administration in the two genders may also have contributed to the difference in DNA methylation observed.

Subsequent to demonstrating cocaine-induced hypo- methylation of the promoter regions of Crem and Fos, we explored the hypothesis that parental cocaine exposure could alter the epigenetic machinery in drug-naive offspring. We subsequently ascertained the inheritance of these epigenetic marks in the offspring. Surprisingly, we found that methylation patterns observed in Crem and Fosb promoter regions in the prefrontal cortex of cocaine-exposed progenitor mice (Fig. 2a and b) were replicated in the same brain region of all groups of non-fostered drug-naive offspring (Fig. 3a and b). This finding showing inheritance of cocaine-induced phenotypes in the mouse brain may have resulted from epigenetic programming in the germlines. Since all variant descendants of cocaine-experienced progenitors displayed similar patterns of methylation status to their parents, it is likely that this pattern of transcriptional induction of Crem/Fosb in the prefrontal cortex stems from the transfer of encoding factors through equal contributions from both germ lines. In contrast to our data, a study by Vassoler et al. [31] delineated heritable phenotypes in rats that self-administered cocaine and reported that male offspring, not females, were resistant to cocaine reinforcement which paralleled activity of Bdnf mRNA and protein levels in the medial prefrontal cortex. The authors further alluded these heritable changes to paternal cocaine experience and increased association of histone H3 and Bdnf promoters in the sperm [31]. A recent study also showed that DNA methylation differences between sperms of addiction-like and non-addiction-like Sprague–Dawley rats were maintained in F0 and F1 descendants and established that transcriptomic changes in addiction-related signalling pathways were linked to epigenetic modifications [22]. Other reports supporting a preponderance of male influence on the epigenetic outcomes of the offspring include studies demonstrating epigenetic inheritance of sperm DNA methylomes in humans [32] and zebrafish [33]. Also, it was reported that the putative binding sites of Crem, among other transcription factors that operate in testicu- lar function were strongly enriched by promoter regions hypomethylated in sperm cells [32].

Nevertheless, our data also showed that the methylome of ME offspring resembled that of their drug-exposed dams. In line with this finding, experiments favouring a female-mediated transfer of epigenetic profiles to the offspring have been documented previously. For instance, Blewitt et al. [34] showed that the yellow coat colour of Agouti offspring mice was more likely a result of maternal rather than paternal inheritance. The notion that paternal epigenetics is the sole inheritance source of off- spring methylomes should therefore be questioned. It is obvious that the process of epigenetic inheritance is complex and that alleles inherited maternally or paternally may be treated differently depending upon the specific gene involved [34]. It further seems that the assessment of methylome profiles in sex cells of progenitor mice and their offspring may be needed to clarify the discrepancies concerning the parental inheritability of epigenetic signatures.

It is well known that early life stressors, such as parental neglect and poor housing conditions, have significant negative effects on the neuropsychological balance of individuals and largely determine vulnerability to the development of behavioural abnormalities including substance abuse [35–37]. Alternatively, there is also evidence that suggest that improvements in the environment may prevent or reverse some of the deleterious effects of early life stressors [38]. In the current study, we examined the impact of early postnatal fostering on the transmitted epigenetic marks of offspring and found that the decreased methylation associated with Crem promoter in the prefrontal cortex of non-fostered pups was reversed by early fostering, especially in fostered FE and ME pups. Since hypomethylation states of Crem (increased gene transcription) are observed with cocaine exposure, a decrease in Crem transcription mediated by fostering may be a strategy to counter the vulnerability offspring inherited from their drug-consuming parents. Previous reports by Miller et al. [13] demonstrated an association between decreased Crem expression levels in the nucleus accumbens, increase impulsive behaviour and increased behavioural sensitivity to intravenous heroin self-administration whereas an opposite response was observed when Crem levels were virally increased in the same brain region. Although these findings suggest that manipulation of Crem levels may have benefit against substance abuse, the contrasting results in the two exper- iments, albeit different experimental protocols and brain regions studied, indicate that further investigations are required before the full therapeutic potential of Crem can be exploited in the management of addictive behaviour.

Weaver [39] previously reported that the early nurturing environment persistently influenced developmental programming of interindividual differences in metabolic and endocrine functions that contribute to emotional and cognitive performance throughout life. Our find- ings showed that all offspring raised by both biological and non-biological MFE parent mice with a history of cocaine exposure were impaired in discriminative learning and thus exhibited memory decline (Fig. 5c and d). This suggests a negative impact of the nurturing medium on cognitive abilities of the drug-naive offspring mice. Besides possible sensitization of the fostered offspring mice to postnatal stimuli [40], a consequential impact of fostering may also be attributed to direct interference of cocaine-induced behavioural alterations of the nursing dams on developmental plastic changes in their offspring. This conclusion is based on a longitudinal study by Johnson et al. [41] that show how maladaptive parental behaviour is associated with increased risk for development of psychiatric disorders among the offspring of parents with and without psychiatric disorders.

Taken together, our data from DNA methylation evaluation showed that some epigenetic marks associated with cocaine exposure were passed down to the next generation of drug-naive descendants. These changes, mirrored by alterations in the epigenetic machinery domicile in the prefrontal cortex, appear to be dynamic as it could be reversed by early postnatal fostering, thus reflecting the interplay between the epigenome and the environment. Finally, an association between cocaine-mediated epigenetic molecular events and impaired cognitive performance of drug-naive offspring mice were also established.

References

LaSalle JM. A genomic point-of-view on environmental factors influencing the human brain methylome. Epigenetics 2011; 6:862–869.

Phillips NLH, Roth TL. Animal models and their contribution to our understanding of the relationship between environments, epigenetic modifications, and behavior. Genes 2019; 10:47.

Rompala GR, Homanics GE. Intergenerational effects of alcohol: a review of paternal preconception ethanol exposure studies and epigenetic mechanisms in the male germline. Alcohol Clin Exp Res 2019; 43:1032–1045.

Richards EJ. Inherited epigenetic variation–revisiting soft inheritance. Nat Rev Genet 2006; 7:395–401.

Novikova SI, He F, Bai J, Cutrufello NJ, Lidow MS, Undieh AS. Maternal cocaine administration in mice alters DNA methylation and gene expression in hippocampal neurons of neonatal and prepubertal offspring. Plos One 2008; 3:e1919.

Gapp K, Jawaid A, Sarkies P, Bohacek J, Pelczar P, Prados J, et al. Implication of sperm rnas in transgenerational inheritance of the effects of early trauma in mice. Nat Neurosci 2014; 17:667–669.

Mashoodh R, Habrylo IB, Gudsnuk KM, Pelle G, Champagne FA. Maternal modulation of paternal effects on offspring development. Proc Biol Sci 2018; 285:20180118.

Lewis CR, Olive MF. Early-life stress interactions with the epigenome: potential mechanisms driving vulnerability toward psychiatric illness. Behav Pharmacol 2014; 25:341–351.

Romens SE, McDonald J, Svaren J, Pollak SD. Associations between early life stress and gene methylation in children. Child Dev 2015; 86:303–309.

Champagne FA. Epigenetic influence of social experiences across the lifespan. Dev Psychobiol 2010; 52:299–311.

Champagne FA. Early adversity and developmental outcomes: interaction between genetics, epigenetics, and social experiences across the life span. Perspect Psychol Sci 2010; 5:564–574.

Ajonijebu DC, Abboussi O, Mabandla MV, Daniels WMU. Differential epigenetic changes in the hippocampus and prefrontal cortex of female mice that had free access to cocaine. Metab Brain Dis 2018; 33:411–420.

Miller M, Ren Y, Szutorisz H, Warren N, Tessereau C, Egervari G, et al. Ventral striatal regulation of CREM mediates impulsive action and drug addiction vulnerability. Mol Psychiatry 2017; 23:1328–

1335.

Levine AA, Guan Z, Barco A, Xu S, Kandel ER, Schwartz JH. CREB- binding protein controls response to cocaine by acetylating histones at the fosB promoter in the mouse striatum. Proc Natl Acad Sci U S A 2005; 102:19186–19191.

Anier K, Malinovskaja K, Aonurm-Helm A, Zharkovsky A, Kalda A. DNA methylation regulates cocaine-induced behavioral sensitization in mice. Neuropsychopharmacology 2010; 35:2450–2461.

Grozdanov PN, Amatullah A, Graber JH, MacDonald CC. Taucstf-64 mediates correct mrna polyadenylation and splicing of activator and repressor isoforms of the cyclic AMP-responsive element modulator (CREM) in mouse testis. Biol Reprod 2016; 94:34.

Brown JR, Ye H, Bronson RT, Dikkes P, Greenberg ME. A defect in nurturing in mice lacking the immediate early gene fosb. Cell 1996; 86:297–309.

Volkow ND, Fowler JS, Wang GJ, Goldstein RZ. Role of dopamine, the frontal cortex and memory circuits in drug addiction: insight from imaging studies. Neurobiol Learn Mem 2002; 78:610–624.

Goldstein RZ, Volkow ND. Dysfunction of the prefrontal cortex in addiction: neuroimaging findings and clinical implications. Nat Rev Neurosci 2011; 12:652–669.

Weissbrod A, Shapiro A, Vasserman G, Edry L, Dayan M, Yitzhaky A, et al. Automated long-term tracking and social behavioural phenotyping of animal colonies within a semi-natural environment. Nat Commun 2013; 4:2018.

Gapp K, Soldado-Magraner S, Alvarez-Sánchez M, Bohacek J, Vernaz G, Shu H, et al. Early life stress in fathers improves behavioural flexibility in their offspring. Nat Commun 2014; 5:5466.

Le Q, Yan B, Yu X, Li Y, Song H, Zhu H, et al. Drug-seeking motivation level in male rats determines offspring susceptibility or resistance to cocaine- seeking behaviour. Nat Commun 2017; 8:15527.

Leger M, Quiedeville A, Bouet V, Haelewyn B, Boulouard M, Schumann- Bard P, Freret T. Object recognition test in mice. Nat Protoc 2013; 8:2531–2537.

Abboussi O, Said N, Fifel K, Lakehayli S, Tazi A, El Ganouni S. Behavioral effects of D3 receptor inhibition and 5-HT4 receptor activation on animals undergoing chronic cannabinoid exposure during adolescence. Metab Brain Dis 2016; 31:321–327.

Paris JJ, Reilley KJ, McLaughlin JP. Kappa opioid receptor-mediated disruption of novel object recognition: relevance for psychostimulant treatment. J Addict Res Ther 2011; S4:007.

Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc

2008; 3:1101–1108.

Irons TD, MacPhail RC, Hunter DL, Padilla S. Acute neuroactive drug exposures alter locomotor activity in larval zebrafish. Neurotoxicol Teratol 2010; 32:84–90.

Horger BA, Iyasere CA, Berhow MT, Messer CJ, Nestler EJ, Taylor JR. Enhancement of locomotor activity and conditioned reward to cocaine by brain-derived neurotrophic factor. J Neurosci 1999; 19:4110–4122.

Zakharova E, Wade D, Izenwasser S. Sensitivity to cocaine conditioned reward depends on sex and age. Pharmacol Biochem Behav 2009; 92:131–134.

Carroll ME, Anker JJ. Sex differences and ovarian hormones in animal models of drug dependence. Horm Behav 2010; 58:44–56.

Vassoler FM, White SL, Schmidt HD, Sadri-Vakili G, Pierce RC. Epigenetic inheritance of a cocaine-resistance phenotype. Nat Neurosci 2013; 16:42–47.

Molaro A, Hodges E, Fang F, Song Q, McCombie WR, Hannon GJ, Smith AD. Sperm methylation profiles reveal features of epigenetic inheritance and evolution in primates. Cell 2011; 146:1029–1041.

Jiang L, Zhang J, Wang JJ, Wang L, Zhang L, Li G, et al. Sperm, but not oocyte, DNA methylome is inherited by zebrafish early embryos. Cell 2013; 153:773–784.

Blewitt ME, Vickaryous NK, Paldi A, Koseki H, Whitelaw E. Dynamic reprogramming of DNA methylation at an epigenetically sensitive allele in mice. Plos Genet 2006; 2:e49.

Ajonijebu DC, Abboussi O, Russell VA, Mabandla MV, Daniels WM. Epigenetics: a link between addiction and social environment. Cell Mol Life Sci 2017; 74:1–13.

Anier K, Malinovskaja K, Pruus K, Aonurm-Helm A, Zharkovsky A, Kalda A. Maternal separation is associated with DNA methylation and behavioural changes in adult rats. Eur Neuropsychopharmacol 2014; 24:459–468.

Andersen SL. Stress, sensitive periods, and substance abuse. Neurobiol Stress 2019; 10:100140.

Ajonijebu DC, Abboussi O, Russell VA, Mabandla MV, Daniels WMU. Epigenetics: a link between addiction and social environment. Cell Mol Life Sci 2017; 74:2735–2747.

Weaver IC. Epigenetic effects of glucocorticoids. Semin Fetal Neonatal Med 2009; 14:143–150.

Vathy I, Slamberová R, Liu X. Foster mother care but not prenatal morphine exposure enhances cocaine self-administration in young adult male and female rats. Dev Psychobiol 2007; 49:463–

473.

Johnson JG, Cohen P, Kasen S, Smailes E, Brook JS. Association of maladaptive parental behavior with psychiatric disorder among parents and their offspring. Arch Gen Psychiatry 2001; 58:453–460.

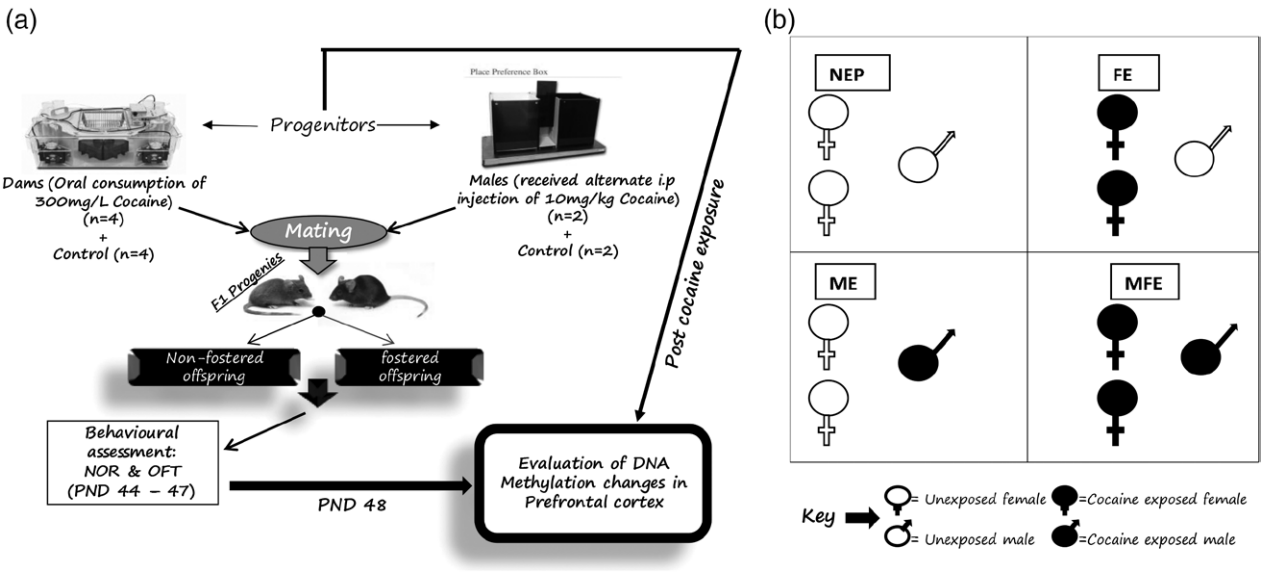

Figure 1. Schematics summarizing parental drug history, mating protocols, and overall behavioural and molecular assay techniques used in this study. (a) Four dams (female progenitors) that experienced 58 days of choice access to cocaine (300 mg/L) solutions via drinking bottles in the IntelliCage apparatus, two sires (male progenitors) that had previously received alternate cocaine (10 mg/kg, i.p) injections over 11 days and their controls (dams, n = 4 and males, n = 2) were paired for mating to produce the F1 generation of naive offspring mice (progenies). Subsequently, DNA methylation in the promoters of Crem and Fosb was assessed in the prefrontal cortex (PFC) of the progenies. (b) Indicate patterns of parental pairing for mating resulting in various groups of offspring used in this study. Shaded and unshaded sex symbols indicate drug-exposed and non-drug-exposed progenitor mice, respectively.

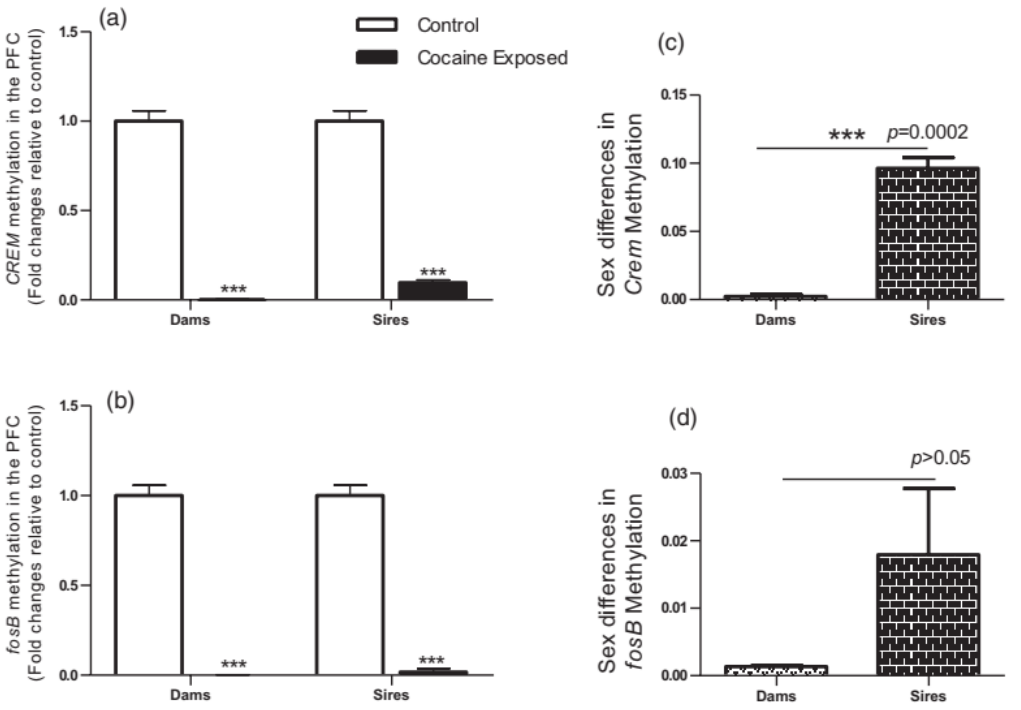

Figure 2. DNA methylation patterns in the prefrontal cortex of parent mice after cocaine experience. The above data show that cocaine exposure caused DNA hypomethylation in *Crem* (a) and *FosB* (b) promoter regions within the prefrontal cortex of both parent mice. The impact of sexual dimorphic effects on *Crem* (c) and *FosB* (d) methylation changes are also presented graphically. Student's *t-test*, ***$P < 0.001$, compared to control or between cocaine treated groups, n = 4. Data shown represents mean ± SD.

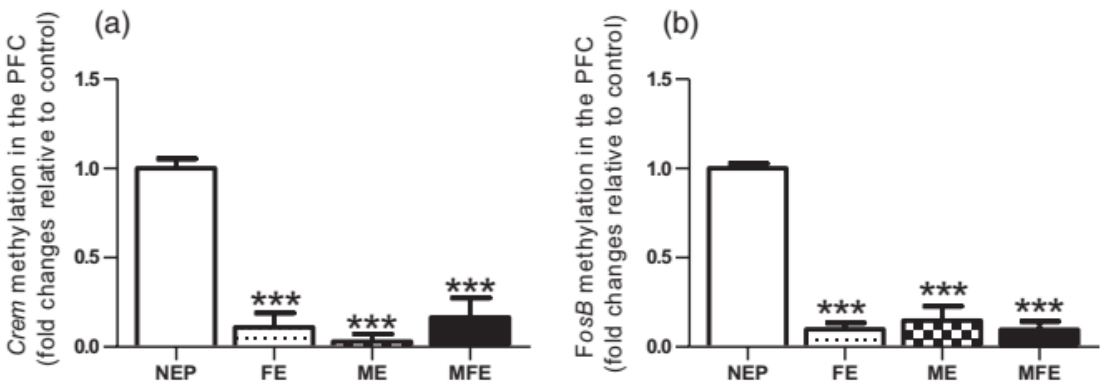

Figure 3. DNA methylation at Crem and FosB promoters in the prefrontal cortex of PND 48 drug-naive non-fostered offspring mice from either non-drug exposed parent (NEP) or cocaine-exposed (FE, ME, MFE) sires/dams. All non-fostered offspring with parental history of cocaine exposure exhibited significant decreases in methylation at Crem (a) and FosB (b) promoters in their prefrontal cortices. One-way ANOVA, Bonferroni's comparison post-hoc test. ***P < 0.0001 (compared to NEP); n = 6/group except non-fostered ME pups with n = 4. Data shown represents mean ± SD. ANOVA, analysis of variance; NEP, non-drug exposed parent; PND, postnatal day.

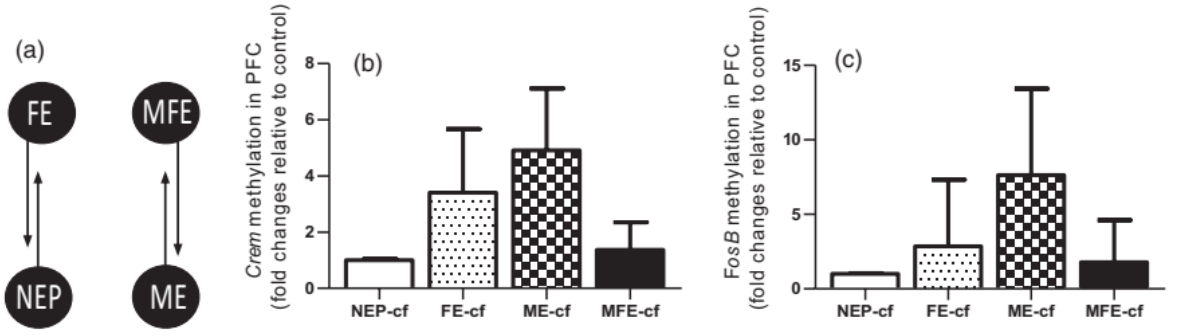

Figure 4. Impact of early postnatal fostering on cocaine-induced epigenetic modifications in the prefrontal cortex. (a) Indicates patterns of cross-fostering amongst variant pups. As shown in the above graphs, there was no evidence of significant alterations in methylation at Crem (b) or fosB (c) promoters in the prefrontal cortex of all fostered pups with or without parental history of cocaine use. One-way ANOVA, Kruskal–Wallis test, *P < 0.05 compared to NEP; n = 6/group except non-fostered ME pups with n = 4. Data shown represents mean ± SD. ANOVA, analysis of variance; NEP, non-drug exposed parent.

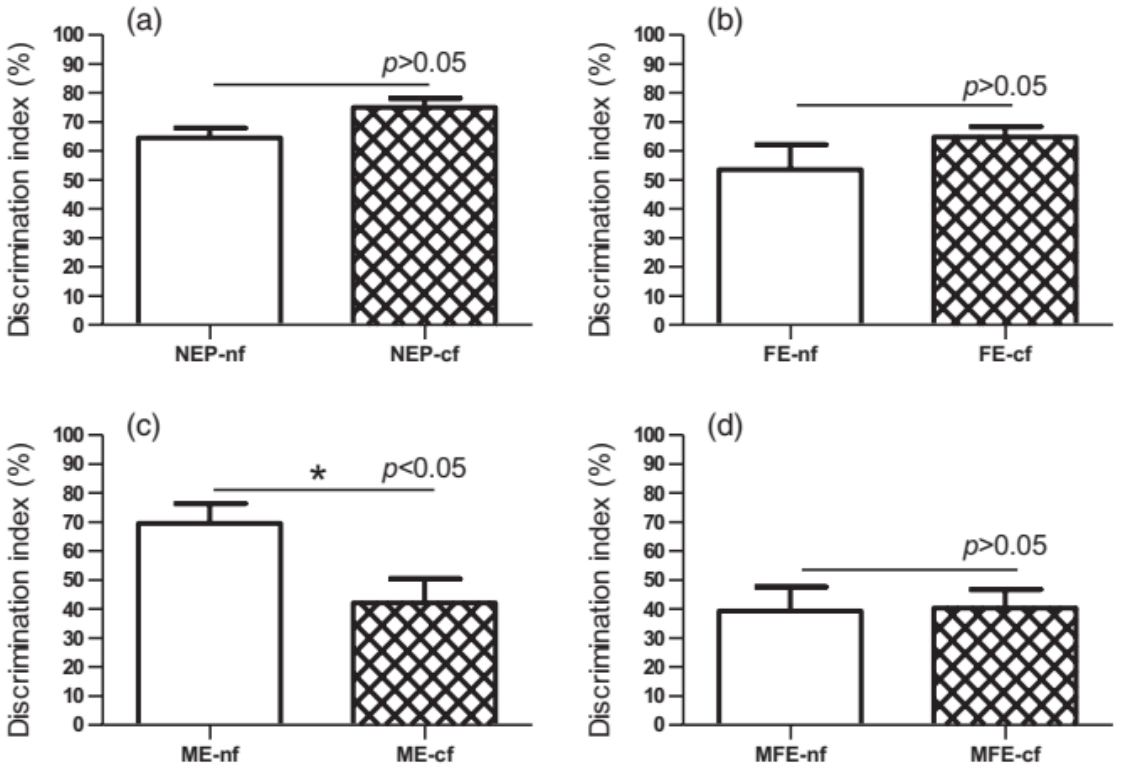

Figure 5. Impact of maternal cocaine exposure and fostering on memory functioning of drug-naive F1 generation offspring mice. Data indicate that object discrimination score was above 50% in both NEP and FE non-fostered groups and maintained in fostered counterpart groups (a and b). ME non-fostered offspring mice also demonstrated a high performance of NOR task which was greatly decreased by fostering (c). Discrimination index remained less than 50% in both non-fostered and fostered MFE offspring (d). Student's *t*-test, **P* < 0.05. n = 6/group except non-fostered ME pups with n = 4. Data shown represents mean ± SD. NEP, non-drug exposed parent; NOR, novel object recognition.

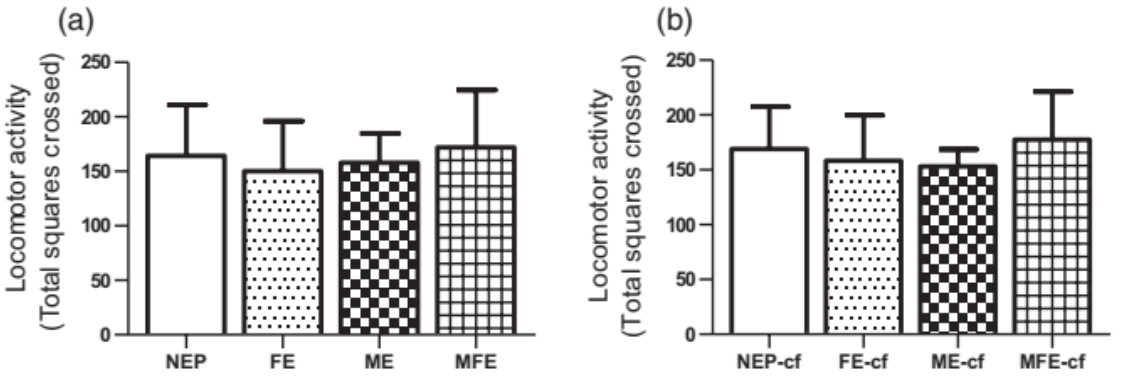

Figure 6. Indicates no change in locomotor activity of either non-fostered (a) or fostered (b) offspring mice that were tested in the OF. One-way ANOVA, $P > 0.05$. n = 6/group except non-fostered ME pups with n = 4. Data shown represents mean ± SD. ANOVA, analysis of variance; OF, open field.